Blind drift correction (*rainSTORM_undrift_auto.m*)

The developed blind drift correction algorithm estimates the overall image drift as the result of sequential linear drifts. The algorithm follows the next four steps.

Separation of the whole images stack into small units

The whole image stack (*N* frames) was divided into 2001 frame long units with overlap of 1000 frames (generating *K=N/1000-1* units). The typical exposure time of dSTORM method is 30 ms that means the data acquisition time of 2001 frames is approximately 60 s. It was assumed that during such a short time, drift can be described via a single $\mathbf{v}_{\mathbf{drift}}$ drift vector.

Estimation of linear drift vector in each unit

The accepted localizations in each 2001 frame long unit were arranged in their precision value order from the highest to the lowest. Then subunits have been formed containing 1000 localizations. Due to this arrangement the first subunit contains the 1000 most precise localizations, the second subunit the second 1000 most precise localizations etc. Next, a pair finding algorithm was applied to pair localizations both inside the subunits and between localizations from different subunits. The algorithm stops after 500,000 accepted pairs. The pair finding and judging algorithm take into consideration the spatial and temporal selection of localizations. For example, localizations captured on subsequent frames with very little lateral drift are disregarded because they presumably belong to the very same blinking event. On other hand, pairs with larger lateral separation than the preset threshold value (distThresh = 2 pixels) are also disregarded since they presumably belong to different structures. Since pair selection algorithm starts with the first subunit, and then evaluates the 2^{nd}, 3^{rd} etc. ones, localizations with higher precession value are taken into consideration first. After selecting 500,000 pairs, the algorithm estimates the drift vector ($\mathbf{v}_{\mathbf{drift}}$) via fitting and maximizing a sum of Gaussian functions on the measured data:

$$\sum_i e^{-\frac{\left(\boldsymbol{d_i} - \boldsymbol{v}_{drift} \bullet \tau_i\right)^2}{2\sigma^2}}$$

where $d_i=(x_{i1}-x_{i2})e_x+(y_{i1}-y_{i2})e_y$ is the distance vector between the two localizations forming the *i*-th pair and *τ_i* is the frame index difference of the localization pair. Because of calculation stability considerations the fitting algorithm first applies large *σ* then reduces its value (1, 0.5 and 0.25). The drift vector in each unit (*K* vectors) is estimated in a similar way.

Interpolation of drift vectors and visualization of drift

Assuming a linear change of $\mathbf{e_x}$ and $\mathbf{e_y}$ components between the *k*-th and *k+1*-th drift vectors, the algorithm estimates the drift vectors for every frames using interpolation. RainSTORM generates a graph to visualize the drift during the measurement (**filename_RemovedDrift**)

Drift correction of the original raw data

Using the estimated drift vectors for each frame, rainSTORM corrects all the original localization coordinates and generates the drift corrected image.